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Changing climate may mitigate the invasiveness risk of non-native salmonids in the Danube and Adriatic basins of the Balkan Peninsula (south-eastern Europe)

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Abstract

Salmonids are an extensively hatchery-reared group of fishes that have been introduced worldwide mainly for their high commercial and recreational value. The Balkan Peninsula (south-eastern Europe) is characterised by an outstanding salmonid diversity that has become threatened by the introduction of non-native salmonids whose potential risk of invasiveness in the region remains unknown and especially so under predicted climate change conditions. In this study, 13 extant and four horizon non-native salmonid species were screened for their risk of invasiveness in the Danube and Adriatic basins of four Balkan countries. Overall, six (35%) of the screened species were ranked as carrying a high risk of invasiveness under current climate conditions, whereas under predicted conditions of global warming, this number decreased to three (17%). Under current climate conditions, the very high risk ('top invasive') species were rainbow

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trout *Oncorhynchus mykiss* and brown trout *Salmo trutta* (*sensu stricto*), whereas under predicted climate change, this was true only of *O. mykiss*. A high risk was also attributed to horizon vendace *Coregonus albula* and lake charr *Salvelinus namaycush,* and to extant Atlantic salmon *Salmo salar* and brook trout *Salvelinus fontinalis*, whose risk of invasiveness, except for *S. fontinalis*, decreased to medium. For the other eleven medium-risk species, the risk score decreased under predicted climate change, but still remained medium. The outcomes of this study reveal that global warming will influence salmonids and that only species with wider temperature tolerance, such as *O. mykiss* will likely prevail. It is anticipated that the present results may contribute to the implementation of appropriate management plans to prevent the introduction and translocation of non-native salmonids across the Balkan Peninsula. Additionally, adequate measures should be developed for aquaculture facilities to prevent escapees of non-native salmonids with a high risk of invasiveness, especially into recipient areas of high conservation value.

Keywords
AS-ISK, extant, fish, horizon, invasive, risk screening

Introduction

Following the exponential increase in recent years in the number of introduced species worldwide (Vilà et al. 2010; Gesundheit and Macias Garcia 2018; Boer et al. 2020; Hughes et al. 2020), biological invasions have become a leading driver of global biodiversity loss (Butchart et al. 2010; Pyšek et al. 2020), posing a serious threat to native biota, including aquatic ecosystems (Piria et al. 2018). In inland waters, freshwater fishes are one of the most frequently introduced groups of organisms (García-Berthou et al. 2005; Cucherousset et al. 2008) that may seriously disrupt ecosystem function through competition, predation, disease, and pest transmission and hybridisation (García-Berthou et al. 2005; Gozlan et al. 2005; Hughes et al. 2020).

Amongst freshwater fishes, salmonids are one of the most widely introduced groups (Buoro et al. 2016), mainly due to their high commercial and recreational value (e.g. Simonović et al. 2015; Piria et al. 2017). As an extensively hatchery-reared group of fishes, salmonids require particular attention since intensive stocking pressure by fishery managers and anglers may threaten the genetic diversity of indigenous salmonid species (Araki and Schmid 2010; Pinter et al. 2019). In this respect, interbreeding between introduced and native salmonids inevitably leads to 'genetic contamination', which may affect either a single population (Crisp 2000) or an entire species, including its evolutionary potential (Pinter et al. 2019). Furthermore, the stocking of salmonids in inland waters is usually done when specimens are ready to consume larger prey; this makes predation one of the principal impacts of salmonids on native aquatic organisms, both vertebrates and invertebrates (Cadwallader 1996; Piria et al. 2020; Čanak Atlagić et al. 2021).

Located in south-eastern Europe, the Balkan Peninsula was a glacial refugium for a large number of endemic species (Bănărescu 2004; Oikonomou et al. 2014) and is currently recognised as one of the world's 35 biodiversity hotspots (Hewitt 2011). In regions that are so important from a conservation perspective, the introduction of new

predators can affect the abundance of native species and increase their risk of extinction (Pyšek et al. 2020), which is of even more concern for valuable and vulnerable endemic species. Examples are Australia and New Zealand, where native galaxiids have been threatened to the brink of extinction by introduced rainbow trout *Oncorhynchus mykiss* and brown trout *Salmo trutta* (McIntosh et al. 1994; McIntosh and Townsend 1995; Glova 2003; Joy et al. 2019).

The exact period of first introduction, re-introduction and translocation of salmonids in the Balkan Peninsula remains unknown, though in the past century these activities have intensified considerably as a result of re-stocking for recreational fishing (Piria et al. 2018). However, besides impacting on the endemic fauna, such practices in the Danube and Adriatic basins of the region may lead to biotic homogenisation of native salmonids amongst which native *Salmo trutta* is known to be particularly threatened (Škraba Jurlina et al. 2020). This taxon is often considered a complex of distinct species concordant with their matching two distinct haplogroups (Bernatchez 2001), whereas the number of species contained within the *Salmo trutta* complex remains debatable (Kalayci et al. 2018; Makhrov and Lajus 2018). However, the introduction of stream-dwelling *Salmo trutta* (*sensu stricto*) of Atlantic origin and of Macedonian trout *Salmo macedonicus* of Adriatic mitochondrial haplotype, originating from the Aegean Basin, has made this unresolved taxonomy even more complicated (Latiu et al. 2020), primarily due to long-term hybridisation (Škraba Jurlina et al. 2020). Additionally, several repeated translocations of salmonids have taken place, mainly from the Danube Basin into the Adriatic Basin of the Balkan Peninsula involving European grayling *Thymallus thymallus* and *Salmo trutta* of Danube origin. Finally, *Hucho hucho* and endemic soft-muzzled trout *Salmo obtusirostris* have also been translocated at different locations within the same basin (Pofuk et al. 2017), with both species flagged as endangered in the IUCN Red List (https://www.iucnredlist.org/).

Previous risk screenings have been carried out for salmonid species partly covering the Danube and Adriatic basins of the Balkan Peninsula (Simonović et al. 2013), as well as for eleven non-native trout species and strains from Serbia (Simonović et al. 2015). However, those screenings did not account for climate change predictions, nor did they include any horizon species, i.e. species present in nearby regions but not yet found in the risk assessment area. A risk screening study accounting for climate change predictions was recently carried out for seven extant and five horizon salmonids (Radočaj et al. 2021), but covered only the northern part of the Danube and Adriatic basins. Hence, the full potential risk posed by extant and horizon salmonid species on the diverse and vulnerable freshwater biota of the Balkan Peninsula remains unknown, especially given climate change predictions of global warming.

To fill the above knowledge gap, the aims of this study were to: (i) identify the translocated and introduced salmonid species of the Danube and Adriatic basins of the Balkan Peninsula; (ii) identify by horizon scanning which non-native salmonid species might enter the Balkan Peninsula in the (near) future from neighbouring countries; and (iii) evaluate the risk of invasiveness of both the identified extant and horizon salmonids under current and future (predicted) climate conditions for the risk assessment area.

Given their extensive use in aquaculture, regular monitoring of the invasiveness of non-native salmonids is crucial to achieve better management of the native freshwater biota of the Balkan Peninsula with the aim of improving appropriate conservation measures.

Methods

Risk assessment area

The risk assessment area includes the Danube and Adriatic basins of Bosnia and Herzegovina, Croatia, Montenegro and Serbia (including Kosovo) (Fig. 1). According to the updated Köppen-Geiger climate map (Rubel et al. 2017), the warm-temperate climate types without dry season *Cfa* and *Cfb* (with warm and hot summer, respectively) are predominant in the risk assessment area and especially in the Danube Basin. Specifically, the *Cfa* type is characteristic of the lower-lying areas of the Danube Basin and of the north-western coastal part of the Adriatic Basin, whereas the *Cfb* type is predominant in the higher-lying continental areas of both basins. The south-eastern coastal part of the Adriatic Basin belongs to the warm-temperate climate types with dry summer *Csa* and *Csb* (warm and hot summer, respectively). Finally, the boreal climate types without dry season *Dfb* and *Dfc* (warm and cold summer, respectively) are found only at the highest elevations of the mountain ranges of the region, namely the Dinaric Alps, Rhodopes, Carpathians and Balkan mountains.

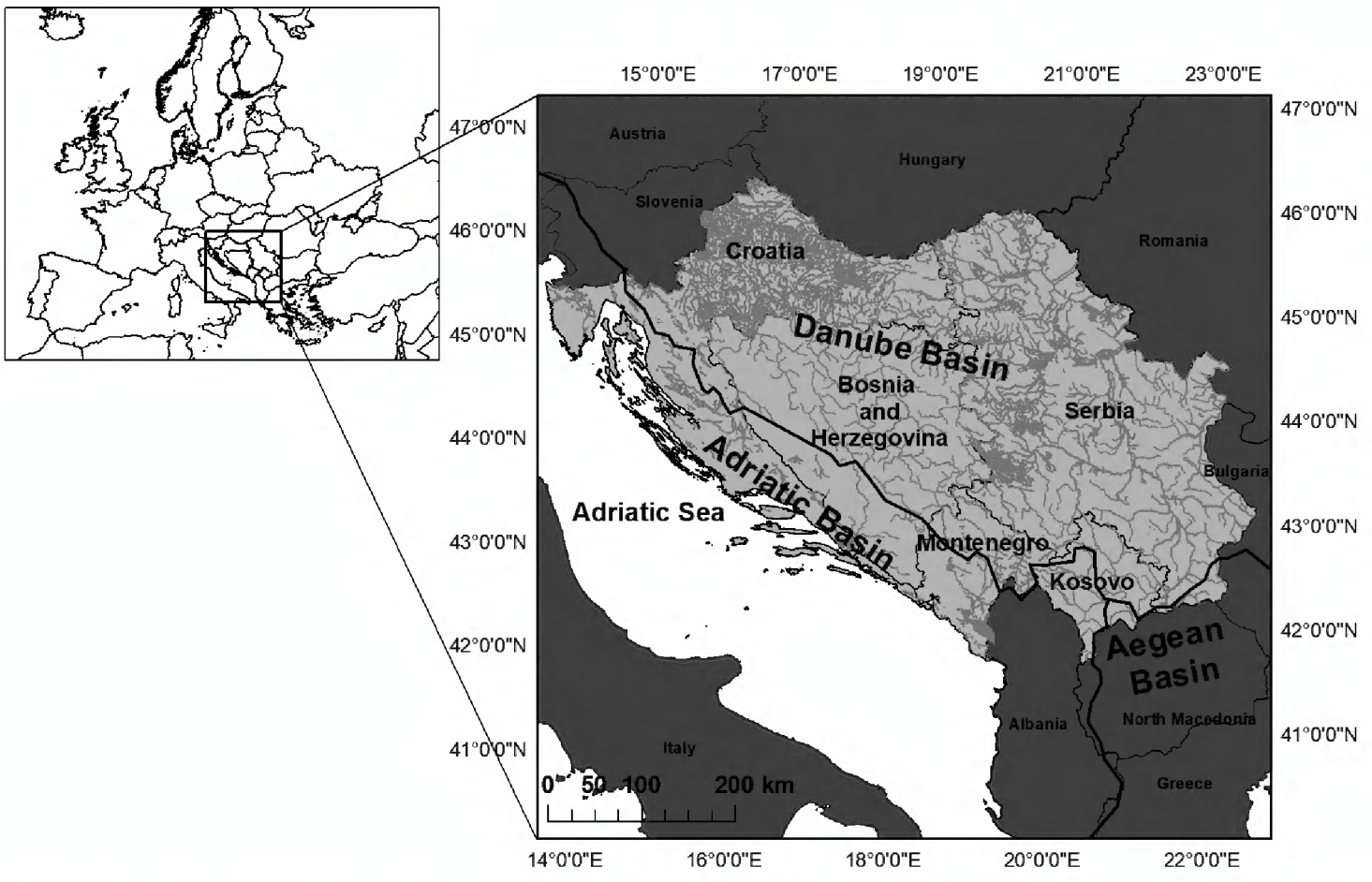

Figure 1. Map of the risk assessment area (Danube and Adriatic Basins of Bosnia and Herzegovina, Croatia, Montenegro and Serbia with Kosovo) and neighbouring countries for evaluating the potential invasiveness of non-native salmonids.

The Danube Basin includes large lowland rivers, amongst which the most important, besides the River Danube, are the River Sava (Bosnia and Herzegovina, Croatia, Serbia) and the River Tisa (Serbia). The largest river of the Adriatic Basin is the River Neretva (Bosnia and Herzegovina, Croatia). Several other large rivers are present, though the main characteristic of the Adriatic Basin's hydrology is the presence of numerous karst-sinking rivers, springs and perennial streams (Jelić et al. 2016; Piria et al. 2017).

The Balkan Peninsula is characterised by a remarkable diversity of native salmonids, especially in the countries of Bosnia and Herzegovina (Škraba et al. 2017), Croatia (Sušnik et al. 2007; Buj et al. 2021), Montenegro (Mrdak et al. 2012) and Serbia (Simonović et al. 2017). At the same time, freshwater salmonid aquaculture in the aforementioned Balkan countries has a long tradition dating back to the late 19th century. The predominantly farmed non-native salmonid species in both the Danube and Adriatic Basins of these countries is *Oncorhynchus mykiss* which, together with *Salmo trutta* (*sensu stricto*) of Atlantic origin, represents the main food fish for inland water re-stocking (Piria et al. 2018). Other salmonid species reared in aquaculture are also found, namely Arctic charr *Salvelinus alpinus*, brook trout *Salvelinus fontinalis* and huchen *Hucho hucho*, although they are mostly used for re-stocking purposes (Kapetanović et al. 2010; Muhamedagić and Habibović 2013; Piria et al. 2018).

Species selection

In total, 17 salmonid species were included as part of the risk screening (Table 1). Selection of the species for screening was according to the following Criteria (where Criteria 1, 2 and 5 are the same as those defined in Piria et al. 2016 and Radočaj et al. 2021):

1. Native species translocated from the Danube Basin to the Adriatic Basin (n = 2: *Thymallus thymallus* and *Salmo labrax*, which also includes the tentative *Salmo taleri*);
2. Native species translocated outside their native range, but within the Danube Basin (n = 1: *Hucho hucho*);
3. Native species translocated outside their native range, but within the Adriatic Basin (n = 1: *Salmo obtusirostris*);
4. Native species translocated from the Aegean Basin to the Danube Basin (n = 1: *Salmo macedonicus*).
5. Non-native species already present and naturalised/acclimatised in one or more drainage basins (n = 8: European whitefish *Coregonus lavaretus*, peled *Coregonus peled*, *Oncorhynchus mykiss*, Ohrid trout *Salmo letnica*, *Salmo salar*, *Salmo trutta* (*sensu stricto*), Arctic charr *Salvelinus alpinus*, brook trout *Salvelinus fontinalis*);
6. Horizon species, i.e. not yet reported, but likely to enter the risk assessment area in the near future (n = 4: lake trout *Salvelins namaycush*, lake charr *Salvelinus umbla*, Chinook salmon *Oncorhynchus tshawytscha*, vendace *Coregonus albula*). These species were selected by using the CABI scanning tool (www.cabi.org/horizonscanningtool) for each country in the risk assessment area separately and by literature searches (e.g. Ventura et al. 2017; Radočaj et al. 2021), including studies in the native language and 'grey' literature.

Risk screening

Risk screening was undertaken using the Aquatic Species Invasiveness Screening Kit (AS-ISK: Copp et al. 2016, 2021), which is available for free download at www.cefas.co.uk/nns/tools. This taxon-generic decision-support tool consists of 55 questions: the first 49 questions comprise the Basic Risk Assessment (BRA) and address the biogeography/invasion history and biology/ecology of the species under screening; the last six questions comprise the Climate Change Assessment (CCA) and require the assessor to predict how future predicted climatic conditions are likely to affect the BRA with respect to risks of introduction, establishment, dispersal and impact. In this study, for the CCA component, local warming scenarios for the Danube and Adriatic Basins of the Balkan Peninsula were used. Accordingly, temperatures are expected to increase from 2030 to 2060 by 1.1–1.7 °C in the Danube Basin (Stagl and Hattermann 2016) and by 1.5–2.5 °C in the Adriatic Basin (Karleuša et al. 2018). In addition, in the Adriatic Basin, an expected decrease in precipitation by 25 mm per decade (5–20% by 2050) would result in a 15% decrease of freshwaters in the Balkan Peninsula (Karleuša et al. 2018). The temperature tolerance ranges for each screened species were searched for in literature, although data were often incomplete and varied depending on the source. Screenings were undertaken on all species initially by four independent assessors (authors AM, IŠ, TK, TR), but with the final screenings based on three assessors (combination 3IA: Vilizzi et al. 2022) (see Results).

To achieve a valid screening, the assessor must provide for each question a response, a level of confidence for the response (see below) and a justification based on literature sources. The outcomes are a BRA score and a (composite) BRA+CCA score, which is obtained after adding or subtracting up to 12 points to the BRA score or leaving it unchanged in case of a CCA score equal to 0. Scores < 1 suggest that the species poses a 'low risk' to become invasive in the risk assessment area, whereas scores ≥ 1 indicate a 'medium risk' or a 'high risk'. The threshold (Thr) value to distinguish between medium-risk (BRA and BRA+CCA score < Thr) and high-risk (BRA and BRA+CCA score ≥ Thr) species for the risk assessment area is obtained by 'calibration' based on the Receiver Operating Characteristic (ROC) curve analysis (see Vilizzi et al. 2022). A measure of the accuracy of the calibration analysis is the area under the curve (AUC) whose values are interpreted as: 0.7 ≤ AUC < 0.8 = acceptable discriminatory power, 0.8 ≤ AUC < 0.9 = excellent, 0.9 ≤ AUC = outstanding (Hosmer et al. 2013). For the species classified as high risk, a distinction was made in this study of the 'very high risk' species, based on an *ad hoc* threshold weighted according to the range of high-risk score values obtained for the BRA and BRA+CCA. Identification of the (very) high-risk species is useful to prioritise allocation of resources in view of a full risk assessment (Copp et al. 2016). This examines in detail the risks of: (i) introduction (entry); (ii) establishment (of one or more self-sustaining populations); (iii) dispersal (more widely within the risk assessment area, i.e. so-called secondary spread or introductions); and (iv) impacts (to native biodiversity, ecosystem function and services, and the introduction and transmission of diseases).

For the ROC curve analysis to be implemented, the species selected for screening must be categorised *a priori* as 'non-invasive' or 'invasive' using literature sources. The

Table 1. Extant and horizon non-native salmonids evaluated for their potential risk of invasiveness in the Danube and Adriatic Basins of Bosnia and Herzegovina, Croatia, Montenegro and Serbia (including Kosovo) – the risk assessment area. The criteria for selection of species are: 1 = Native species translocated from the Danube Basin to the Adriatic Basin; 2 = Native species translocated outside their native range but within the Danube Basin; 3 = Native species translocated outside their native range, but within the Adriatic Basin; 4 = Native species translocated from the Aegean Basin to the Danube Basin; 5 = Non-native species already present and naturalised/acclimatised in one or more drainage basins; 6 = Horizon species, i.e. not yet reported but likely to enter the risk assessment area in the near future. For extant species, details about the native distribution area are provided including the location and year of introduction. For all species, the *a priori* categorisation outcome into Non-invasive and Invasive is provided, based on a multi-tiered protocol (after Vilizzi et al. 2022) relying on FishBase (www.fishbase.org), the Global Invasive Species Database (GISD: www.iucngisd.org), the Centre for Agriculture and Bioscience International Invasive Species Compendium (CABI: www.cabi.org/ISC), the Invasive and Exotic Species of North America list (IESNA: www.invasive.org) and a Google Scholar literature search whenever applicable. N = no impact/threat; Y = impact/threat; '–' = absent; n.e. = not evaluated (but present in database); n.a. = not applicable.

Taxon name	Common name	Criterion	Distribution area			*A priori* categorisation					
			Native	Introduced	Year	FishBase	GISD	CABI	IESNA	GScholar	Outcome
Extant											
Coregonus lavaretus	European whitefish	5	Northern Europe	Plitvice lakes, Peruča Reservoir, River Cetina	1937	N	–	–	–	N	Non-invasive
Coregonus peled	peled	5	Northern Europe	Plitvice Lakes, Peruča reservoir, River Cetina	1937	–	–	–	–	N	Non-invasive
Hucho hucho	huchen	2	Europe	Rivers Đetinja, Jerma, Nišava, Mlava, Moravica	2001	N	–	–	–	N	Non-invasive
Oncorhynchus mykiss	rainbow trout	5	North America	Vlasina Reservoir	1792	Y	Y	Y	Y	n.a.	Invasive
Salmo labrax	Black Sea salmon	1	Eurasia	Rivers Gacka, Vrijeka	1948	N	–	–	–	N	Non-invasive
Salmo letnica	Ohrid trout	5	Europe, Lake Ohrid	Vlasina Reservoir	1950	N	n.e.	–	–	N	Invasive
Salmo macedonicus	Macedonian trout	4	Central Europe	River Jerma	2000	N	–	–	–	N	Non-invasive
Salmo obtusirostris	soft-muzzled trout	3	Europe, Adriatic Basin	River Žrnovnica	1970s	N	–	–	–	N	Non-invasive
Salmo salar	Atlantic salmon	5,6	Northern Europe	Krka Estuary, rivers Sava and Drava	1980	N	N	Y	–	n.a.	Invasive
Salmo trutta (*sensu stricto*)	brown trout	5	Western Europe	Rivers Gacka, Gradac, Vratna	1970	Y	Y	Y	Y	n.a.	Invasive
Salvelinus alpinus	Arctic charr	5	Northern Europe	Plitvice lakes, River Neretva, Peruča accumulation, Lake Kokin Brod	1963	N	–	–	–	N	Non-invasive
Salvelinus fontinalis	brook trout	5	North America	Plitvice lakes, River Neretva, Peruča accumulation, Lake Kokin Brod	1960	Y	Y	Y	–	n.a.	Invasive
Thymallus thymallus	grayling	1	Eastern Europe	Rivers Cetina, Gacka, Istria, Neretva, Rude	1960	N	–	–	–	N	Non-invasive
Horizon											
Coregonus albula	vendace	6	–	–	–	N	Y	–	–	n.a.	Invasive
Oncorhynchus tshawytscha	chinook salmon	5	–	–	–	–	N	–	–	N	Non-invasive
Salvelinus namaycush	lake charr	6	–	–	–	N	Y	Y	–	n.a.	Invasive
Salvelinus umbla	Alpine charr	6	–	–	–	N	–	–	–	N	Non-invasive

a priori categorisation of species was implemented as per Vilizzi et al. (2022) (Table 1). Confidence levels in the responses to questions in the AS-ISK are ranked using a 1–4 scale and, based on the confidence level (CL) allocated to each response, a confidence factor (CF) is obtained as:

$$CF = \sum(CL_{Qi})/(4 \times 55) \ (i = 1, \ldots, 55)$$

where CL_{Qi} is the CL for Q*i*, 4 is the maximum achievable value for confidence (i.e. very high: see above) and 55 is the total number of questions comprising the AS-ISK questionnaire (Vilizzi et al. (2022). The CF ranges from a minimum of 0.25 (i.e. all 55 questions with confidence level equal to 1) to a maximum of 1 (i.e. all 55 questions with confidence level equal to 4). Based on all 55 Qs of the AS-ISK questionnaire, the 49 Qs comprising the BRA and the six Qs comprising the CCA, the CF_{Total}, CF_{BRA} and CF_{CCA} are respectively computed.

Implementation of the ROC curve analysis followed the protocol described in Vilizzi et al. (2022), with the true/false positive/negative outcome distinction not applied to the medium-risk species, as they can be either included or not into a full (comprehensive) risk assessment depending on priority and/or availability of financial resources. The ROC curve fitting was in two steps. Firstly, separate ROC curves were generated for each of the four independent assessors and differences amongst the resulting four AUCs were statistically tested (Mann-Whitney *U*-statistic, $\alpha = 0.05$; applet StAR available at http://melolab.org/star/home.php: Vergara et al. 2008). As differences between assessor-specific AUCs were found, in the second step, a single ROC curve was generated, based on the average scores of those assessors whose AUC was above the acceptable discriminatory power. Following ROC analysis, the best threshold value that maximises the true positive rate and minimises the false positive rate was determined using Youden's *J* statistic; whereas the 'default' threshold of 1 was set to distinguish between low-risk and medium-risk species. Fitting of the ROC curve was with package pROC (Robin et al. 2011) for R x64 v.4.0.5 (R Core Team 2021) using 2000 bootstrap replicates for the confidence intervals of specificities, which were computed along the entire range of sensitivity points (i.e. 0 to 1, at 0.1 intervals). Differences in outcome scores and CF between components (BRA and BRA+CCA) and assessors (AM, IŠ, TK, TR for the scores; AM, IŠ, TR for the CF) were tested with permutational ANOVA, based on a two-factor design with factors Component and Assessor crossed and both fixed. Analysis was implemented in PERMANOVA+ for PRIMER v.7, with normalisation of the data and using a Bray-Curtis dissimilarity measure, 9999 unrestricted permutations of the raw data and with statistical effects evaluated at $\alpha = 0.05$, including *a posteriori* pair-wise comparisons in case of significance.

Results

Across all four assessors (Fig. 2): the BRA scores ranged from 0 to 38.0, with mean = 18.3, median = 16.8 and 5% and 95% CI (confidence interval) = 3.1 and 36.5; the BRA+CCA scores ranged from –6.0 to 48.0, with mean = 13.5, median = 11.5 and 5% and 95%

CI = –2.0 and 33.0. The mean BRA score was significantly higher than the mean BRA+CCA score (18.3 ± 9.6 SE vs. 13.5 ± 11.8) and the overall scores (i.e. BRA and BRA+CCA) for assessor AM (21.7 ± 10.9) were significantly higher than those for the

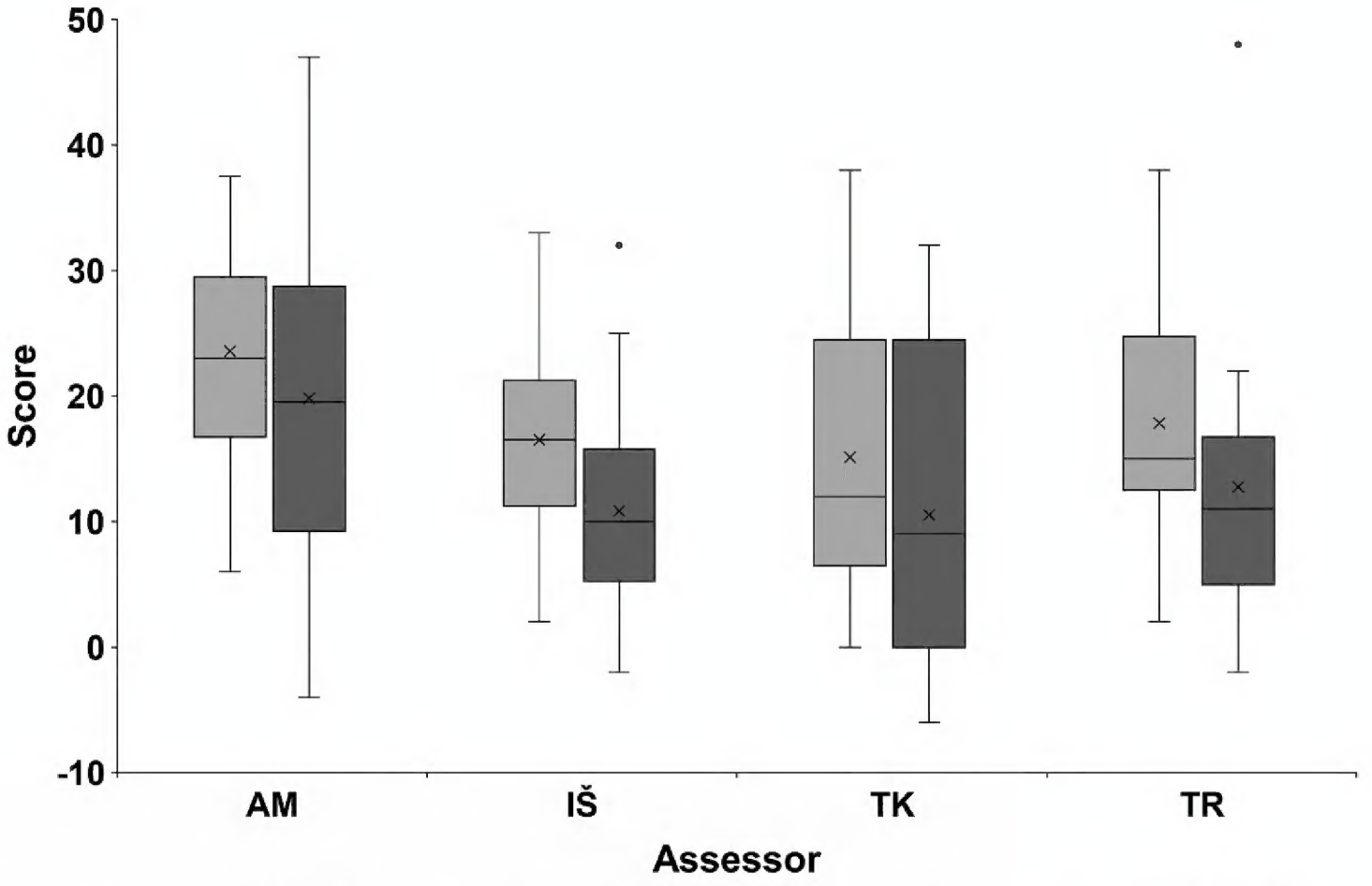

Figure 2. Box-and-whisker plots showing the Aquatic Species Invasiveness Screening Kit (AS-ISK) outcome scores (Basic Risk Assessment, BRA: light grey; BRA + Climate Change Assessment, BRA+CCA: dark grey) for the four assessors (AM = Ana Marić; IŠ = Ivan Špelić; TK = Tamara Kanjuh; TR = Tena Radočaj) screening the non-native salmonids for the risk assessment area (see Fig. 1).

Table 2. Permutational ANOVA results for the Aquatic Species Invasiveness Screening Kit (AS-ISK) outcome scores and for the confidence factor (CF) of the non-native salmonids screened for the risk assessment area. Component = BRA, BRA+CCA (see Table 3).

Source of variation	df	MS	$F^{\#}/t$	$P^{\#}$
Scores				
Component	1	6.431	7.177	0.009
Assessor	3	4.578	5.109	0.002
AM vs. IŠ	1	–	3.402	**< 0.001**
AM vs. TK	1	–	3.228	**0.003**
AM vs. TR	1	–	2.522	**0.014**
IŠ vs. TK	1	–	0.343	0.734
IŠ vs. TR	1	–	0.717	0.476
TK vs. TR	1	–	0.928	0.352
Component × Assessor	3	0.045	0.050	0.984
Residual	128	0.896		
CF				
Component	1	10.540	24.515	**< 0.001**
Assessor	2	23.664	55.040	**< 0.001**
AM vs. IŠ	1	–	5.058	**< 0.001**
AM vs. TR	1	–	10.111	**< 0.001**
IŠ vs. TR	1	–	5.604	**< 0.001**
Component × Assessor	2	0.929	2.162	0.123
Residual	96	0.430		

other assessors (13.7 ± 8.8 for IŠ, 12.8 ± 11.9 for TK, 15.3 ± 10.3 for TR). However, there was no interaction term, indicating that the BRA and BRA+CCA scores did not differ between each other depending on the assessor (Table 2).

There were differences in AUCs between AM and TK ($P < 0.01$), whose AUC had a much lower value (i.e. 0.6143, hence below acceptable discriminatory power) compared to the AUCs from AM, IŠ and TR (i.e. 0.9143, 0.8000 and 0.8786, respectively, hence with excellent to outstanding discriminatory power). As a result, the BRA score outcomes from TK were removed from subsequent analyses and the threshold value was computed, based on the mean BRA scores from AM, IŠ and TR. The ROC curve resulted in an AUC of 0.9286 (0.7810–1.0000 95% CI), which indicated outstanding discriminatory power. Youden's J provided the threshold of 19.25, which was used for calibration of the risk outcomes. Accordingly, based on the BRA scores, the threshold allowed the distinction of medium-risk species with scores within the interval [1, 19.25[from high-risk species with scores within [19.25, 68]; based on the BRA+CCA scores, the threshold allowed the distinction of medium-risk species with scores within the interval [1, 19.25[from high-risk species with scores within [19.25, 80]. Low-risk species had BRA scores within [–20, 1[and BRA+CCA scores within [–32, 1[(see Table 2; combined AS-ISK report in Suppl. material 1). Using the above threshold:

- Based on the BRA outcome scores (Table 3): six (35.3%) species were classified as high risk and eleven (64.7%) as medium risk. Amongst the seven species categorised *a priori* as invasive, six were true positives (*Coregonus albula*, *Oncorhynchus mykiss*, *Salmo salar*, *Salmo trutta*, *Salvelinus fontinalis*, *Salvelinus namaycush*). Of the eleven medium-risk species, ten were *a priori* non-invasive and one invasive.
- Based on the BRA+CCA outcome scores, hence after accounting for climate change predictions (Table 3): three (17.6%) species were classified as high risk, 13 (76.5%) as medium risk and one (5.9%) as low risk (*Hucho hucho*). Amongst the *a priori* invasive species, three were true positives (*Oncorhynchus mykiss*, *Salmo trutta*, *Salvelinus fontinalis*) and, amongst the ten species categorised *a priori* as non-invasive, one was a truer negative (*Hucho hucho*). Of the 13 medium-risk species, nine were *a priori* non-invasive and four invasive.

The highest-scoring species (BRA and BRA+CCA scores > 30, taken as an *ad hoc* 'very high risk' threshold) were *Oncorhynchus mykiss* and *Salmo trutta* for both the BRA and BRA+CCA and *Oncorhynchus mykiss* only for the CCA. The CCA resulted in a slight increase in the BRA score for only one species (*Oncorhynchus mykiss*), in no change for another species (*Salmo macedonicus*) and in a decrease for the remaining 15 species (Table 3).

The mean CF_{Total} was 0.707 ± 0.017 SE, the mean CF_{BRA} 0.720 ± 0.018 and the mean CF_{CCA} 0.593 ± 0.020. Across the three assessors (i.e. AM, IŠ and TR), the mean CF_{BRA} was significantly higher than the mean CF_{CCA} and the overall CF (i.e. for the BRA and CCA) for assessor AM (0.792 ± 0.112) was significantly higher than that for assessors IŠ (0.663 ± 0.135) and TR (0.515 ± 0.147), which also differed significantly. However, there was no interaction term, indicating that CF_{BRA} and CF_{CCA} did not differ between each other depending on the assessor (Table 3).

Table 3. Risk outcomes for the non-native salmonids screened with AS-ISK for the risk assessment area. For each species, the following information is provided: *a priori* categorisation of invasiveness (N = non-invasive; Y = invasive: see Table 1); BRA and BRA+CCA scores with corresponding risk outcomes (M = Medium; H = High; VH = Very high based on *ad hoc* threshold of 30: see text for details) and classification (Class: TN = true negative; TP = true positive; '–' = not applicable as medium-risk: see text for details); difference (Delta) between BRA+CCA and BRA scores; confidence factor (CF) for all 55 questions of the AS-ISK (CF_{Total}), for the 49 BRA questions (CF_{BRA}) and for the six CCA questions (CF_{CCA}). Risk outcomes are based on a threshold of 19.25 and computed as: L, within the interval [–20, 1[, M [1, 19.25[, H [19.25, 30[and VH [30, 68] for the BRA; L [–32, 1[, M [1, 19.25[, H [19.25, 30[and VH [30, 80] for the BRA+CCA (note the reverse bracket notation indicating in all cases an open interval).

Taxon name	*A priori*	BRA			BRA+CCA			Delta	CF		
		Score	Outcome	Class	Score	Outcome	Class		Total	BRA	CCA
Coregonus albula	Y	19.3	H	TP	10.0	M	–	–9.3	0.64	0.64	0.58
Coregonus lavaretus	N	18.7	M	–	14.0	M	–	–4.7	0.74	0.77	0.54
Coregonus peled	N	14.5	M	–	10.5	M	–	–4.0	0.73	0.75	0.57
Hucho hucho	N	10.0	M	–	0.0	L	TN	–10.0	0.75	0.76	0.61
Oncorhynchus mykiss	Y	33.7	VH	TP	42.3	VH	TP	8.7	0.86	0.88	0.72
Oncorhynchus tshawytscha	N	17.5	M	–	13.5	M	–	–4.0	0.70	0.70	0.68
Salmo labrax	N	19.2	M	–	15.2	M	–	–4.0	0.70	0.71	0.64
Salmo letnica	Y	15.8	M	–	11.2	M	–	–4.7	0.64	0.65	0.60
Salmo macedonicus	N	18.3	M	–	17.0	M	–	–1.3	0.57	0.58	0.44
Salmo obtusirostris	N	8.0	M	–	2.0	M	–	–6.0	0.72	0.72	0.75
Salmo salar	Y	22.2	H	TP	17.5	M	–	–4.7	0.65	0.68	0.44
Salmo trutta	Y	32.8	VH	TP	26.8	H	TP	–6.0	0.76	0.78	0.58
Salvelinus alpinus	N	19.2	M	–	13.2	M	–	–6.0	0.72	0.73	0.61
Salvelinus fontinalis	Y	29.8	H	TP	24.5	H	TP	–5.3	0.76	0.79	0.53
Salvelinus namaycush	Y	24.5	H	TP	15.8	M	–	–8.7	0.66	0.67	0.57
Salvelinus umbla	N	9.8	M	–	3.8	M	–	–6.0	0.63	0.63	0.63
Thymallus thymallus	N	14.8	M	–	8.8	M	–	–6.0	0.80	0.82	0.58

Discussion

Risk outcomes

In this study, the risk of invasiveness of 17 salmonids was determined with a very high level of accuracy (cf. discriminatory power), based on independent assessors. According to the threshold value of 19.25, based on the BRA, only six (35%) species were classified as carrying a high risk of invasiveness for the risk assessment area, whereas based on the BRA+CCA, this number decreased to three (17%). A similar decrease in score for salmonids under predicted climate change scenarios has been observed for Croatia and Slovenia (Radočaj et al. 2021), Turkey (Yoğurtçuoğlu et al. 2021) and even for regions with colder climate ranging from humid continental to sub-arctic as found in the West Siberian Plain (Interesova et al. 2020). In this study, the mean CF was lower for the CCA compared to the BRA, which agrees with other AS-ISK applications (e.g. Bilge et al. 2019; Interesova et al. 2020; Radočaj et al. 2021) and reflects the uncertainty in climate change predictions generally due to a dearth of literature for several of the screened species. On the contrary, for a species like *Oncorhynchus mykiss* for which the impact of climate change has been largely investigated (e.g. Benjamin et al. 2013; Stanković et al.

2015), the CF value for the CCA was the highest amongst all species in this study (0.72: Table 3), similar to screenings for this species in other risk assessment areas compared to other salmonids (e.g. Tarkan et al. 2017: 0.74; Moghaddas et al. 2021: 0.77).

Of the screened species, seven were found to pose a high to very high risk of invasiveness for the RA area under current climate conditions (BRA). However, after accounting for predicted climate change conditions (CCA), for four of these species, the risk of invasiveness decreased from high to medium (Table 3). Specifically, only *Oncorhynchus mykiss* was classified as very high risk for both the BRA and BRA+CCA, whereas *Salmo trutta*, which was classified as very high risk for the BRA, became of high risk after accounting for climate change. Both species belong to the List of the 100 World's Worst Invasive Alien Species (GISD 2021), likely as a result of their vagility, life history, phenotypic plasticity, broad water temperature tolerance and highly adaptive behaviour, as documented worldwide (Crowl et al. 1992; Hardy 2002; Hasegawa 2020). Finally, *Salvelinus fontinalis* was the only species classified as high risk for both the BRA and BRA+CCA.

Oncorhynchus mykiss is a top predator whose negative effects in its introduced range resulting from its carnivorous diet have been documented worldwide (Skelton 1987; Young et al. 2010; Juncos et al. 2013). In the risk assessment area, this species' impact is mostly reflected on the endemic minnow-like fishes (Zupančič et al. 2008), which has led to the near-extinction of *Telestes metohiensis* from the River Ljuta near Dubrovnik in Croatia (Piria et al. 2016). In its native range, *O. mykiss* is an anadromous species that can tolerate high salinities and a wide range of water velocities (Leitwein et al. 2017), and for this reason it is found even in catches of commercial fishers from the Adriatic Sea (M. Piria, pers. obs.). Although it is generally presumed that *O. mykiss* cannot establish viable populations in the risk assessment area, there are some documented cases of its reproduction dating back to the early 20th century in Slovenia (Franke 1913; Mršić 1935), the early 1970s in Croatia (MacCrimmon 1971), plus several more recent reports (e.g. Stanković et al. 2015; Mihinjač et al. 2019). In addition, there is evidence of reproduction in a population of *O. mykiss* in the Međimurje area (P. Simonović, pers. obs.), in the rivers of southern Croatia (D. Zanella, pers. obs.) and in southern Greece on the Island of Crete (Koutsikos et al. 2012; Stoumboudi et al. 2017).

Salmo trutta (*sensu stricto*) is one of the most attractive recreational salmonids in the risk assessment area that, however, poses a major threat to the native salmonids because of genetic contamination. Introgression of alien Atlantic haplotypes into the indigenous *Salmo labrax* and *Salmo obtusirostris* gene pool has already been documented (Simonović et al. 2014, 2015; Tošić et al. 2016; Škraba et al. 2017; Kanjuh et al. 2020, 2021; Škraba Jurlina et al. 2020), with the size of the intact native populations of these two species still remaining unknown. Although the score for *Salmo trutta* decreased as a result of the CCA, this species remains at high risk for the risk assessment area probably because of its dispersal mechanisms, which are often deliberate through farming and stocking for recreational purposes. In this respect, the first stocking of this species in the risk assessment area occurred in early 20th century and has become quite intensive in recent times (Simonović et al. 2014; Piria et al. 2018).

Salvelinus fontinalis is a valuable species for angling both in the risk assessment area and worldwide (Lenhardt et al. 2011; CABI 2021). There are known cases where the presence of introduced *S. fontinalis* has negatively affected populations of native amphibians in France and Spain (Orizaola and Braña 2006). In addition, this species has been found to overlap its diet with native *Salmo trutta* populations in southern France with which it also interferes in terms of reproductive success and hybridisation (Cucherousset et al. 2007, 2008), though resulting in sterile offspring (Hisar et al. 2003). There is evidence that *S. fontinalis* may exert detrimental impacts on native *Salmo trutta* in Sweden, leading to extinction of some native populations (Spens et al. 2007). All of this confirms that this species carries several undesirable life-history traits, which is in line with its high-risk ranking.

The three *a priori* invasive species *Coregonus albula*, *Salmo salar* and *Salvelinus namaycush* gained a high risk of invasiveness under current climate conditions (cf. BRA) whereas under the BRA+CCA, their risk became medium. *Coregonus albula* and *Salmo salar* are characterised by behavioural and developmental plasticity, which makes them capable to react and potentially adapt to variation in environmental conditions. However, there are limitations to these capacities, especially over short periods of time (Garcia de Leaniz et al. 2007; Muir et al. 2013; Karjalainen et al. 2015, 2016). In this regard, water temperature is fundamental in regulating fish physiology and environmental variation during development can play a crucial role in generating variability in offspring through phenotypic plasticity (Little et al. 2020). Migratory fishes, such as *S. salar*, are particularly vulnerable to warming environments as the appropriate time of transition between habitats is fine-tuned to specific environmental cues (Crozier et al. 2008), with the success of these transition periods having consequences on subsequent survival. *Salvelinus namaycush* is known to migrate to deep cold-water habitats and generally occupies temperatures within its optimum range (10 °C ± 2 °C: Plumb and Blanchfield 2009). This is aside from brief forays into shallow warm-water habitats to forage (Morbey et al. 2006) or to avoid limiting oxygen conditions at high depths (Guzzo and Blanchfield 2017). Therefore, increasing temperatures would drastically affect populations of *S. namaycush* by reducing suitable summer thermal habitats and by increasing exposure to sub-optimal temperatures and thermal stress (Ficke et al. 2007; Guzzo and Blanchfield 2017), thereby limiting growth and condition (Plumb et al. 2014; Guzzo et al. 2017).

Salmo letnica was the only *a priori* invasive species found to carry a medium risk of invasiveness likely due to its low dispersal mechanism traits, but also to the scarce data available to answer the AS-ISK questions about 'undesirable traits' (see Copp et al. 2016). *Coregonus lavaretus*, *Coregonus peled*, *Oncorhynchus tshawytscha*, *Salmo labrax*, *Salmo macedonicus*, *Salmo obtusirostris*, *Salvelinus alpinus*, *Salvelinus umbla* and *Thymallus thymallus* were all classified as medium-risk for both the BRA and BRA+CCA, with the risk for *Hucho hucho* becoming low after accounting for the CCA. The latest outcome is expected because *Hucho hucho* is an already threatened species due to the relatively long period of time to reach maturity during which it is intolerant of pollution and damming (Weiss and Schenekar 2016).

Climate change

As cold-water species, salmonids are likely to be strongly affected by climate change. An increase in temperature and a decrease in precipitation can directly influence water levels in rivers and lakes (e.g. Schindler 2001), with consequent changes in other water-related characteristics, such as food amount and composition, acidity and other chemical parameters (Cochrane et al. 2009). These changes could trigger a range of negative responses in salmonid fishes and especially in those species with complex life-histories consisting of several developmental stages (Crozier et al. 2008). Studies on the potential effects of climate change on salmonids have shown complex behavioural responses in *Oncorhynchus mykiss* exposed to different seasonal temperatures, acidity, nitrogen and food supply (Morgan et al. 2001; Ficke et al. 2007). Higher air temperatures could affect productivity or even cause mortality in aquaculture ponds via increased water temperatures, especially for salmonids with a narrow water temperature range (Cochrane et al. 2009). Although most salmonid ponds have a flow-through system with frequent water exchange that can mitigate increases in temperature, climate change can affect water regime by causing drought or flood events. With global warming, more precipitation events occurs as rainfall instead of snowfall, snow melts earlier and there is increased runoff and risk of flooding in early spring, but increased risk of drought in summer, especially in continental areas (Trenberth 2011; Karleuša et al. 2018). Overall, warming conditions in temperate regions across the Globe will probably not only narrow the distribution of wild salmonid stocks, but also reduce the number of appropriate sites for salmonid farming (Cochrane et al. 2009).

Implications for aquaculture

The most suitable streams for salmonid farming in the risk assessment area are in Montenegro, western Croatia and Bosnia-Herzegovina because of the presence of extensive areas with higher altitudes and boreal climate conditions. Interestingly, all salmonid farming in the risk assessment area and surrounding countries (i.e. Albania, Bulgaria, North Macedonia) is based on non-native species with *Oncorhynchus mykiss* being predominant (Koutsikos et al. 2019), followed by *Salmo trutta* (*sensu stricto*) (Piria et al. 2018). Other non-native farmed salmonid species include *Coregonus lavaretus*, *Coregonus peled*, *Salvelinus alpinus* and *Salvelinus fontinalis*. However, due to current aquaculture strategies and proposed diversification of species, farmers are trying to diversify their production with more profitable species (Ministry of Agriculture 2020). For example, in Croatia, there is an attempt to introduce *Salmo salar* in aquaculture. However, because of: (i) Regulation (EU) no. 1143/2014 of the European Parliament and of the Council on the prevention and management of the introduction and spread of invasive alien species and (ii) Council Regulation (EC) No 708/2007 of 11 June 2007 concerning the use of alien and locally absent species in aquaculture, plus (iii) national law, the introduction of new species for aquaculture in countries which are part of the European Union is becoming increasingly difficult (Piria et al. 2017, 2021a).

Overall, it is advised that non-native species introductions should be brought to a minimum or avoided altogether and that every introduction of a new species should

be conducted only after a full risk assessment (e.g. Tarkan et al. 2020, 2022), because any new fish species in aquaculture carries a risk of escape (De Silva 2012). However, in countries that are not part of the EU (e.g. Bosnia-Herzegovina and Serbia), hence do not need to abide by the above EU regulations (Piria et al. 2021a), the introduction of new species in aquaculture relies only on local laws and regulations, which do not include any risk assessment. If an escape eventually occurs, monitoring programmes could be used as an early-warning system before the new species becomes established. This is especially true of large river systems, where an introduced species can be detected early so that any adverse impact can be contained (Radočaj et al. 2021). Furthermore, accidental escapees from fish farms can be a source of pathogen transmission to wild stocks (Krkošek et al. 2007; Rosenberg 2008) and this is another important threat still understudied (Wood et al. 2021). Despite tight trade measures, established customs and quarantine methods and protocols related to transboundary aquatic diseases in the Member States of the EU, introductions of new pathogens into aquaculture are still occurring (Peters et al. 2018; Pofuk 2021). Similarly, biosecurity regulations in the countries of the risk assessment area are well-developed for aquaculture, although inspection and control do not function well, whereas regulations remained completely undeveloped for the purposes of open-water re-stocking (Pofuk 2021).

Management actions

In the countries of the risk assessment area, freshwater fishing is regulated by different fisheries acts. For example, in Serbia, stocking is limited by law to native species only (Official Gazette 2018) with penalties for misdemeanours as in the case of stocking occurring not under professional control (Official Gazette 2005). Similarly, in Croatia, Montenegro and Bosnia-Herzegovina, local fisheries acts and mandatory management plans regulate stocking activities and prevent or limit possibilities for the stocking of non-native species (Vehanen et al. 2020; Piria et al. 2021a). Despite existing legislation in the countries of the risk assessment area to prevent stocking of rivers and streams with non-native fish, there are still possible pathways that mediate new (unauthorised) introductions by anglers and escapees from aquaculture (Britton et al. 2011; Cerri et al. 2018). These pathways of introduction are especially important for salmonids because of their value for local aquaculture and angling (Simonović et al. 2015). In particular, in the risk assessment, area stocking with non-native species is still possible into isolated water bodies without access to inland waters, where such species are already naturalised and have been present for a long time. Such introductions are still legally supported and prescribed in anglers' management plans. The best example of this practice is in the karst region of the River Lika in Croatia, where more than 90% of fishes are of non-native origin (i.e. mostly translocated from another basin but within the same country) and anglers' management plans are based on re-stocking with 'native' fish species, which in fact have never been native to the region (Piria et al. 2021b). On the contrary, in other connected river systems and inland waters of the risk assessment area, this practice is prohibited, so that all recent introductions with non-native fishes (if any) are considered illegal and there is no available information on such practices.

Possibly the most challenging (and still unrecognised) problem for the Balkan Peninsula is the legal stocking of salmonid streams with *Salmo trutta* (*sensu stricto*), which poses a threat to native genetic integrity (Kanjuh et al. 2020; Piria et al. 2020; Buj et al. 2021). *Salmo trutta* (*sensu stricto*) is considered a native species by law in all countries of the risk assessment area. This is because management plans for salmonid re-stocking require performing obligatory stocking by *S. trutta*, although without any specification of which lineage. In the risk assessment area, only *S. trutta* (*sensu stricto*) is found for aquaculture and there are no producers of native *Salmo* sp. for re-stocking (Piria et al. 2020). If anglers do not re-stock based on this management plan, a misdemeanour report by the inspectorate will follow. Thus, decision-makers cannot prohibit re-stocking with a species that is prescribed to be re-stocked, even if it belongs to a different lineage. Clearly, the problem of genetic contamination is still not well recognised by decision-makers and stakeholders and currently, in the risk assessment area, *S. trutta* (*sensu stricto*) interacts with *Salmo obtusirostris*, *Salmo labrax* and *Salmo macedonicus* by changing their original gene pool.

Control and containment of introduced salmonids, once established, is the only advisable approach, since eradication is virtually impossible in river systems and large lakes (Britton et al. 2011). However, containment (e.g. by artificial barriers preventing migration) and control (e.g. by gillnets and electrofishing) can be very costly endeavours and may sometimes conflict with local legislation, thereby making them not feasible across the risk assessment area. A possible solution for the control of established populations of salmonids in the long term could be to encourage anglers to remove non-native salmonids from the wild. Another solution could be the obligation by fishing clubs to use exclusively native lineages of salmonids for stocking local river systems, although in this case it would be necessary to encourage farmers to produce indigenous salmonids. Decision-makers may follow for example the farming of *Hucho hucho* in Bosnia and Herzegovina and in Slovenia for stocking in rivers where the species is indigenous (Andreji and Stráňai 2013; Muhamedagić and Habibović 2013) or of marble trout *Salmo marmoratus* by banning stocking of *Salmo trutta* (*sensu stricto*). This could be achieved by revising fishing regulations for anglers and genetically testing brood stock from hatcheries for stocking phenotypic and pure young-of-the-year *S. marmoratus* (Berrebi et al. 2022).

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Supplementary material 1

Combined AS-ISK report including the 68 screenings for the 17 salmonid species screened for the Danube and Adriatic basins of Bosnia and Herzegovina, Croatia, Montenegro and Serbia (including Kosovo)

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